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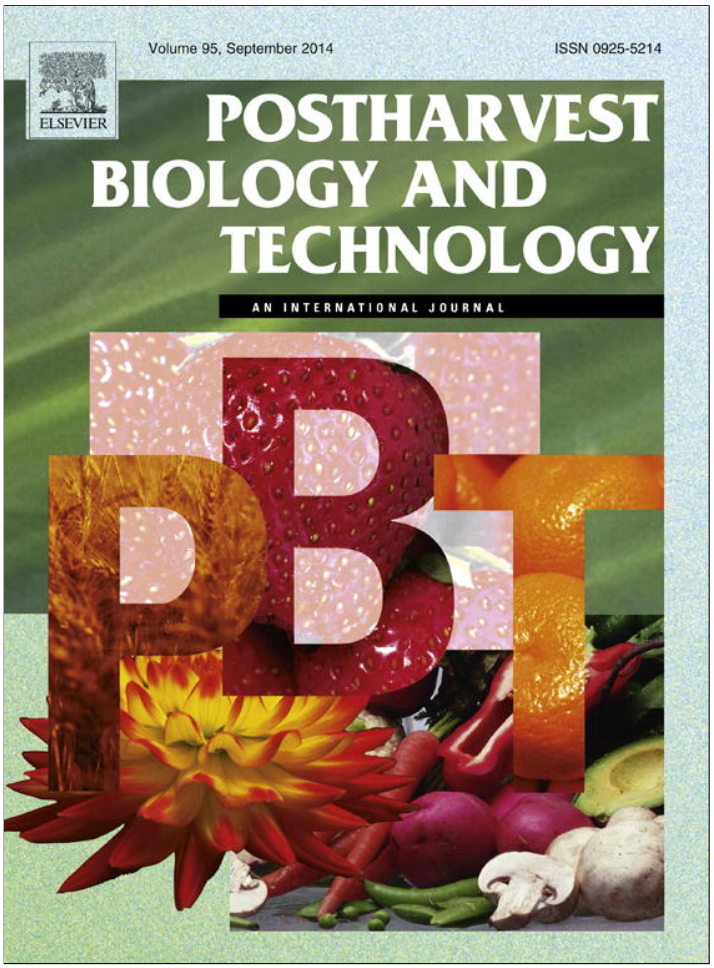

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UV-C inactivation of *Escherichia coli* and dose uniformity on apricot fruit in a commercial setting☆

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ABSTRACT

A UV-C treatment system (two treatment chambers connected by an inclined belt to rotate apricots between chambers) was tested in a commercial setting. *Escherichia coli* ATCC 25922, used as a surrogate for *E. coli* O157:H7 to determine the system's antimicrobial efficacy, was inoculated onto fruit surfaces at a population of 6.8 log CFU/fruit. UV-C dosage was evaluated by attaching film dosimeters to six fixed locations on each apricot. Results suggested that reduction of inoculated *E. coli* ATCC 25922 populations on the apricot fruit by UV-C treatment was small (only 0.5–0.7 logs). There were large variations in UV-C doses among varying apricot surface locations. Approximately 1/3 of apricots had individual surfaces receiving less than 0.2 kJ m^{-2} UV-C exposure, even though fruit received, on average, more than 1 kJ m^{-2}. Low reductions of *E. coli* may be attributed, in part, to non-uniform UV-C exposure. This study demonstrates the need to use a fruit rotation device more capable of delivering uniform UV-C dosage to the surface of apricots for inactivating bacteria in a commercial setting.

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1. Introduction

The United States' Food Safety Modernization Act requires food producers to implement control measures to eliminate hazards, including microbial human pathogens, that are reasonably likely to occur (US-FDA, 2011). Soft fruits, such as tree-ripe peaches and apricots, cannot tolerate washes and are packed in dry condition by packers and producers due to their advanced ripeness and softness (Crisosto et al., 1995; Crisosto and Valero, 2008). Therefore, producers and packers of soft fruits need to use control methods other than washes to achieve the necessary bacterial reduction.

Ultraviolet C (UV-C) radiation at a wavelength range of 100–280 nm damages DNA of bacterial pathogens and inactivates pathogens. Bialka and Demirci (2007) used UV-C treatments for decontamination of *Escherichia coli* and *Salmonella enterica* on blueberries. After 60 s of pulsed UV treatment, maximum reductions of 4.3 and 2.9 log CFU/g for *Salmonella* and *E. coli*, respectively, were reported. Yaun et al. (2004) found that UV-C at an ambiguous dose (without given exposure time) resulted in 3.3 and 2.8 log reductions of *E. coli* O157:H7 on apples and green leaf lettuce, respectively, and 2.2 log reduction of *Salmonella* spp. on tomatoes.

One challenge for commercial application of UV-C on fresh produce is how to ensure dose uniformity due to irregular shape and overlap of fruits and vegetables during UV-C treatment. In addition, for any processing technology to be successfully implemented by the food industry, the technology has to be validated in a commercial setting to verify that the technology achieves the required microbial reductions under the conditions typically encountered in the production environment. For tests to be conducted in commercial establishments, surrogates of pathogenic bacteria have to be used. The objective of the present study was to investigate the effectiveness of the UV-C in inactivating *E. coli* inoculated on apricots, and evaluating the UV-C dose uniformity that fruit received in a commercial setting.

2. Materials and methods

2.1. Source of fruit

Robada apricots at the commercial maturity stage were harvested from an orchard in central Washington State one day prior to UV-C treatment.

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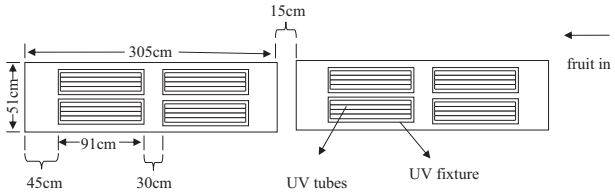

Fig. 1. Diagram of commercial UV-C treatment chambers.

2.2. UV-C treatment system

The UV-C treatment system consisted of two 305 cm UV-C treatment chambers (Fig. 1). Each treatment chamber was placed on the top of a trough, which housed the conveyer belts (~51 cm width). The two UV-C chambers were connected with an inclined belt (8 cm (height) × 15 cm (length)) which was used to rotate fruit between the two UV-C chambers. Fruit were placed in one end of the UV-C chamber and carried by the conveyor belts into the UV-C chambers. There were four UV-C fixtures in each treatment chamber with two fixtures opposite and parallel to each other. The distance between the two parallel fixtures was about 8 cm. Each UV-C fixture consisted of a metal hood (~19 cm in width) hosting four UV-C light tubes (Model GML005, 13.8 W, ~91 cm long, American Air and Water, Hilton Head Island, SC, USA). The light fixtures were located 45 cm from each end of the chamber and the distance between the two sets of fixtures was 30 cm within each chamber. The distance from the light tubes to the conveyer belt was about 14 cm. The temperature of the packing house was about 15 °C.

2.3. Suitability of E. coli ATCC 25922 as a surrogate for E. coli O157:H7

The suitability of *E. coli* ATCC 25922 as a surrogate for *E. coli* O157:H7 was tested in a laboratory setting. *E. coli* ATCC 25922 was obtained from American Type Culture Collection (Manassas, VA, USA). Five strains of *E. coli* O157:H7 (RM 6535, RM 7386, O6FOO475 and RM 1484) were provided by Dr. Robert Mandrell of USDA, ARS, Western Regional Research Center. The preparation and inoculation of apricot fruit with *E. coli* O157:H7 and *E. coli* ATCC 25922 were performed similarly as described earlier (Yun et al., 2013). Inoculated fruit were treated with UV-C in a custom-built UV-C chamber (Yun et al., 2013) for 15 or 90 s at an intensity of ~10 W m^{-2}. During treatment, the fruit were rotated using a roller grill machine (Great Northern Popcorn Com., Mancelona, MI, USA) in which the heater was disconnected and the speed of the roller rotation was increased to 0.6 s^{-1}. After the UV treatment, bacteria were recovered by massaging each fruit in 150 mL of sterile buffered peptone water for 30 s. Then the suspension was serially diluted and plated on tryptic soy agar (TSA) containing 100 mg L^{-1} rifampicin and Sorbitol MacConkey Agar (SMAC) (Difco, Sparks, MD, USA). Colonies were enumerated after 24 h of incubation at 37 °C. There were 3 fruit for each treatment, and experiments were repeated three times.

2.4. Reduction of E. coli 25922 by UV-C in a commercial setting

Medium size (68 ± 8 g) Robada apricot fruit were inoculated individually via dipping fruit into the *E. coli* ATCC inoculum (300 mL) for 30 s with agitation. Fruit were removed from the inoculum and dried for 2–3 h on racks in a biological hood. The inoculated fruit were placed onto the UV-C conveyor and run through the UV treatment chambers at two speeds: 20 and 40 s. The control fruit samples were not exposed to UV-C light. The two UV-C treatment times were chosen based on the measurements of UV-C light intensity in the chambers and our previous study on the reduction of human pathogens (Yun et al., 2013) which demonstrated that 0.15–0.20 kJ m^{-2} of UV was generally required to achieve a 1 log reduction of *E. coli* O157:H7 and *Salmonella* spp. on apricots. The calculated UV-C exposure would achieve more than 1 log reduction of *E. coli*. After treatment, bacteria were recovered as described earlier, serially diluted and plated on TSA with 100 mg L^{-1} rifampicin and on SMAC. Plates were incubated for 24 h at 37 °C, and colonies were counted. The experimental design resulted in 4 replicates per treatment with three fruit assessed in each of the four replicates. After the UV-C treatment, the UV-C system was left on for 10 min followed by sanitization with 200 mg L^{-1} chlorine and 70% ethanol. No *E. coli* was found using the swab test.

2.5. UV dose uniformity of fruit using film dosimeters

Three sizes of Robada apricots with average weights of 97, 73 and 58 g for large, medium and small, respectively, were used in the study. Radiachromic films (FWT-60-00, size 1 cm × 1 cm, average thickness 43.5 μm, Far West Technology, Goleta, CA, USA) were attached to six different surface locations on each of 60 apricots (20 apricots for each size). These locations were tip, stem, C1, C2, C3 and C4. The tip is the end opposite to the stem side, while the stem was considered the cavity around the stem. C2 is the apricot suture (the line running from stem to tip) area, and C4 is the cheek area opposite the suture. C1 and C3 are the cheek areas on opposite sides about 90° from C2 and C4, respectively. The fruit were then processed through the UV-C chambers (20 s). Absorbance of each film was measured at 510 nm using a radiachromic reader (Model 92, Far West Technology). The UV-C doses received by the fruit were calculated based on A510 nm from a standard curve established using the same films receiving known doses of UV-C.

2.6. Experimental design and statistical analysis

Data were subjected to General Linear Model (GLM) procedures using SAS ver. 9.12 (SAS Institute, Raleigh, NC, USA). The effects of UV dose were analyzed using the Least Significant Difference ($P < 0.05$).

3. Results and discussion

3.1. Reduction of E. coli populations

UV-C treatment for 15 s reduced populations of *E. coli* O157:H7 composites by 1.3 and 1.7 logs CFU/fruit when assessed on TSA and SMAC, respectively (Fig. 2). With a 90 s UV-C treatment, populations of *E. coli* O157:H7 were reduced by 1.4 and 1.8 log CFU/fruit on TSA and SMAC, respectively. Reductions (1.0–1.8 log CFU/fruit) of *E. coli* ATCC 25922 by UV-C were not significantly different from those of *E. coli* O157:H7 at either dose or growing medium, suggesting that ATCC 25922 could be used as a surrogate for *E. coli* O157:H7 in UV-C treatments on the surface of apricots.

In the commercial setting, *E. coli* ATCC 25922 populations were reduced by only about 0.5 log CFU/fruit when enumerated on both TSA and SMAC after a 20 s UV-C treatment (Fig. 3). Reductions were 0.7 and 0.6 log CFU following 40 s treatments, as enumerated on both TSA and SMAC, respectively; however, the reductions were not statistically significant as variations among the replicates were high.

3.2. Dose uniformity on fruit

The UV-C intensity in the commercial UV-C chambers was measured. In the middle part of each chamber, the UV-C intensities ranged from 10.3 to 16.8 W m^{-2}. Low UV intensity was observed between the two sets of UV-C fixtures as well as at either end of

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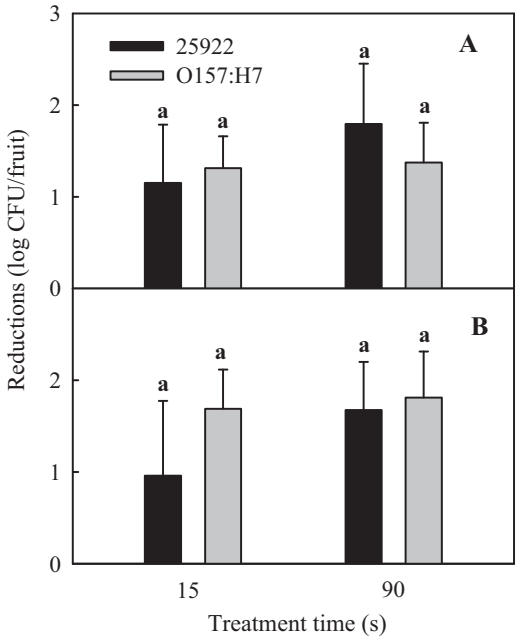

Fig. 2. Comparison of *E. coli* ATCC 25922 with *E. coli* O157:H7 in response to UV-C exposure. Bacteria were enumerated on TSA (A) and SMAC (B). Bars with the same letter are not significantly different ($P < 0.05$). Vertical bars represent standard deviations.

the UV-C chambers. In the chambers, there was little variation in UV-C intensity transversely compared to the longitudinal variation.

Film dosimeters were used to measure radiation doses received by each fruit. Following UV-C exposure, film dosimeters turned blue (Fig. 4), where the degree of blueness correlated to the UV doses received. Results showed that there were large variations in UV doses among the six locations tested on each fruit (Fig. 5). Fruit size did not affect the UV-C dose uniformity. UV-C intensity in the chambers varied depending on location, and, as indicated by the variations in UV doses among six locations on the same fruit, some areas of the fruit surface received 10 times greater doses of UV radiation than other areas. On average, the tip area of the apricots received the lowest doses, regardless of fruit size (Fig. 6), while the average dose (calculated from 6 locations) that each fruit received was 1.27 kJ m^{-2}. Fruit used

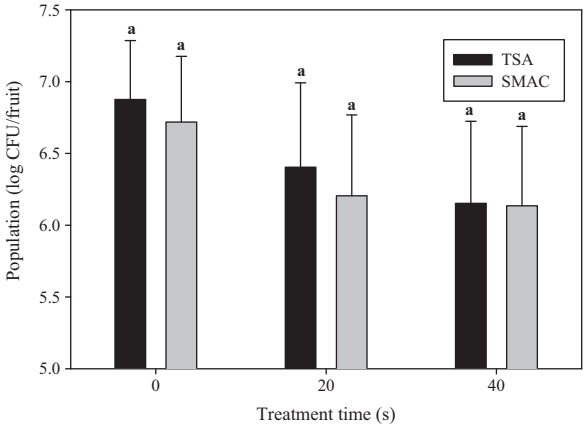

Fig. 3. Effect of UV-C treatment at a commercial facility on *E. coli* population inoculated on apricots. Apricots inoculated with *E. coli* ATCC 25922 were treated with UV-C for 20 and 40 s. Vertical bars represent standard deviations.

Fig. 4. Apricot fruit with attached film dosimeters after UV-C treatment.

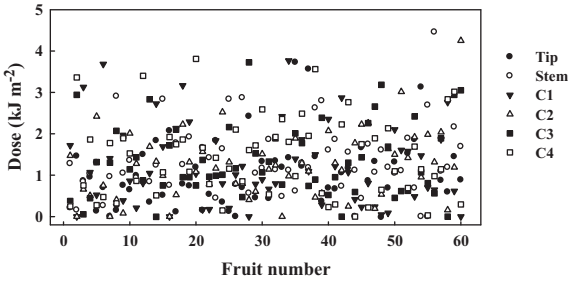

Fig. 5. UV-C dose uniformity calculated from film dosimeter on different surface areas of fruit. Sixty fruit with attached film dosimeters traversed UV-C treatment chambers in a commercial facility. Six film dosimeters were attached on each fruit at different locations (tip, stem, C1, C2, C3 and C4). Tip is the end opposite the stem. Stem is the cavity around the stem. C2 is the suture (the line running from stem to tip) area. C4 is the cheek area opposite the suture. C1 and C3 are the cheek areas other than C2 and C4. After treatments, the absorbance at 510 nm of each film was measured. Doses were calculated based on a standard curve.

for *E. coli* reduction were treated with UV for 20 and 40 s, corresponding to radiation doses of 1.27 and 2.54 kJ m^{-2}, respectively. Even though, on average, each fruit received UV doses more than 1 kJ m^{-2}, one third of the fruit (20 out of 60) had part of the fruit surface receiving less than 0.2 kJ m^{-2} of UV-C.

Our results suggest that UV-C, used in the commercial setting, only achieved 0.5–0.7 log CFU/fruit inactivation of surrogate *E. coli* ATCC 25922. When a modified rotation device was used in the laboratory setting, UV-C treatment of 15 s reduced *E. coli* ATCC 25922

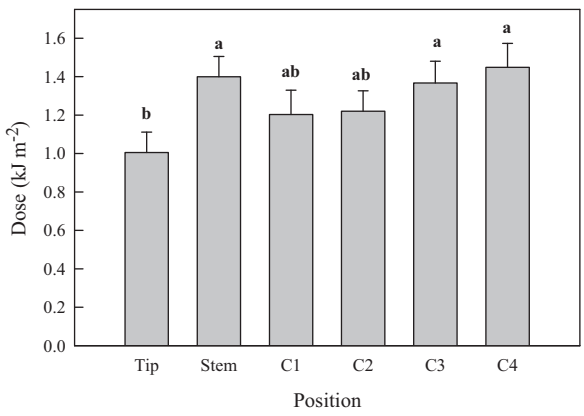

Fig. 6. Average UV-C doses on different areas of apricot fruit. See text or Fig. 5 for the description of C1–C4.

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populations by 1.0–1.2 log CFU/fruit and *E. coli* O157:H7 populations by 1.3–1.8 log CFU/fruit, with a calculated average UV dose received of 1.12 kJ m^{-2}, which was slightly less than the average dose (1.27 kJ m^{-2}) the fruit received in the commercial setting. The UV-C doses (on average) received by the fruit in the commercial setting should be high enough to achieve a minimum of 1 log reduction of *E. coli* ATCC 25922; nevertheless, reductions in the commercial setting were only 0.5–0.7 log CFU/fruit, which may be attributed to lower UV-C doses received by certain surfaces of some fruit. Efforts need to be made to rotate fruit during the UV-C treatment such that fruit receive uniform UV-C exposure in order to achieve a higher reduction of pathogens. Furthermore, studies may be conducted to evaluate whether fruit can synthesize antibacterial compounds after UV-C treatment as suggested by Mercier et al. (2001) showing that UV-C induced disease resistance in bell peppers.

These results showed that the UV-C treatments achieved insignificant (about 0.5–0.7 log CFU/fruit) reductions of *E. coli* ATCC 25922 populations on apricots, which may be a function of the large variation in UV-C doses that fruit received at different surface locations. An improved method for rotating fruit may be needed to achieve higher reduction of *E. coli*. Overall, these results suggested that a UV-C system needs to be optimized in order to enhance microbial safety of soft fruits in conjunction with other good manufacturing practices and good agricultural practices.

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